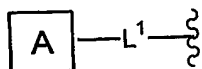


## CLAIMS

What is claimed is:

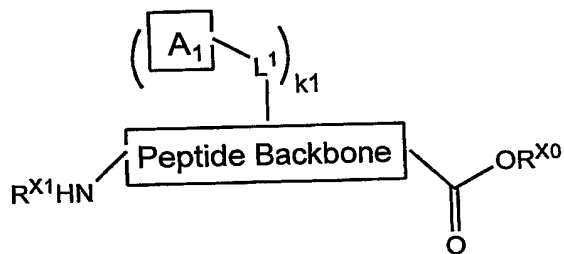
1. A method for preparing a polyfunctionalized peptide comprising a peptidic backbone made up of four or more amino acids wherein two or more non-adjacent amino acids are independently substituted with a moiety having the structure:



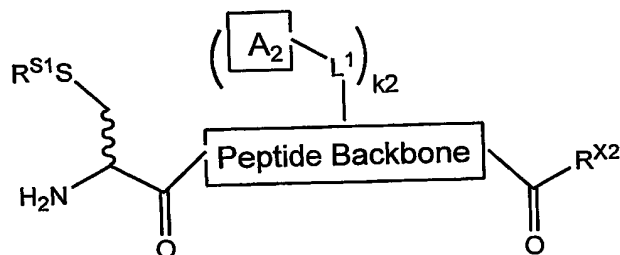
with the proviso that the peptide sequence between any two consecutive, non-adjacent, amino acids bearing a  $A-L^1$ - moiety comprises at least one cysteine residue;

- 10 wherein the method comprises a step of:

reacting a peptide acyl donor comprising a peptidic backbone made up of two or more amino acids wherein said peptide acyl donor has the structure:



with a peptide amine acceptor having the structure:



15

under suitable conditions to effect ligation;

wherein  $k1$  and  $k2$  are independently integers between 1 and about 20;

each occurrence of  $A$ ,  $A_1$  and  $A_2$  is independently an aliphatic, heteroaliphatic, aromatic, heteroaromatic, aryl, heteroaryl or a pharmaceutically useful group or entity;

20

$R^{S1}$  is a sulfide protecting group;

$R^{X0}$  is a group such that the moiety  $-\text{C}(=\text{O})\text{OR}^{X0}$  can be made to undergo ligation with the peptide amine acceptor;

each occurrence of  $L^1$  is independently a substituted or unsubstituted, linear or branched, cyclic or acyclic, saturated or unsaturated aliphatic or heteroaliphatic moiety;

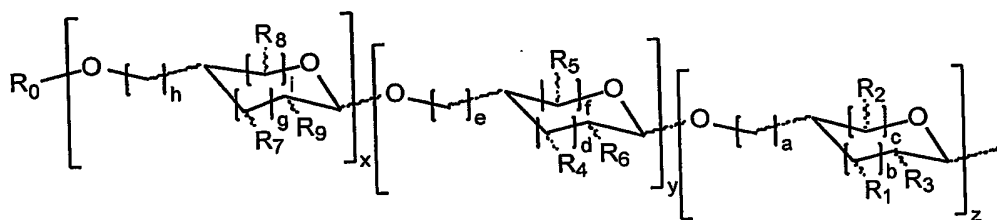
$R^{X1}$  is hydrogen, alkyl, acyl, aromatic, heteroaromatic, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), a nitrogen protecting group, an amino acid or a protected amino acid;

$R^{X2}$  is  $-OR^{X2a}$  or  $-NR^{X2b}R^{X2c}$ , wherein  $R^{X2a}$  is hydrogen, alkyl, aromatic, heteroaromatic, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), a carboxylic acid protecting group, an amino acid or a protected amino acid; and  $R^{X2b}$  and  $R^{X2c}$  are independently hydrogen, alkyl, aromatic, heteroaromatic, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), a nitrogen protecting group, an amino acid or a protected amino acid.

2. The method of claim 1, wherein each occurrence of A, A1 and A2 is independently a pharmaceutically useful group or entity.

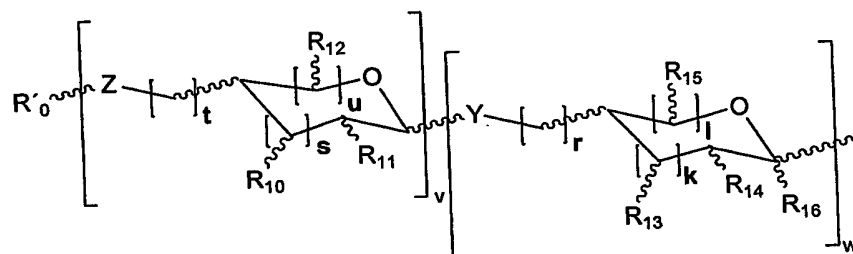
3. The method of claim 1, wherein each occurrence of A, A1 and A2 is independently a biomolecule, a small molecule, a macromolecule or a diagnostic label.

4. The method of claim 1, wherein each occurrence of A, A1 and A2 is independently a carbohydrate determinant having the structure:



wherein a, b, c, d, e, f, g, h, i, x, y and z are independently 0, 1, 2 or 3, with the proviso that the x, y and z bracketed structures represent furanose or pyranose moieties and the sum of b and c is 1 or 2, the sum of d and f is 1 or 2, and the sum of g and i is 1 or 2, and with the proviso that x, y and z are not simultaneously 0; wherein  $R_0$  is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl

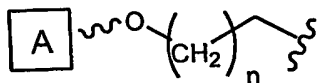
group; wherein each occurrence of  $R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8$  and  $R_9$  is independently hydrogen, OH,  $OR^i$ ,  $NHR^i$ ,  $NHCOR^i$ , F,  $CH_2OH$ ,  $CH_2OR^i$ , a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of  $R^i$  is independently hydrogen, CHO,  $COOR^{ii}$ , or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group or a saccharide moiety having the structure:



wherein Y and Z are independently NH or O; wherein k, l, r, s, t, u, v and w are each independently 0, 1 or 2; with the proviso that the v and w bracketed structures represent furanose or pyranose moieties and the sum of l and k is 1 or 2, and the sum of s and u is 1 or 2, and with the proviso that v and w are not simultaneously 0; wherein  $R'_0$  is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of  $R_{10}, R_{11}, R_{12}, R_{13}, R_{14}$  and  $R_{15}$  is independently hydrogen, OH,  $OR^{iii}$ ,  $NHR^{iii}$ ,  $NHCOR^{iii}$ , F,  $CH_2OH$ ,  $CH_2OR^{iii}$ , or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of  $R_{16}$  is hydrogen, COOH,  $COOR^{ii}$ ,  $CONHR^{ii}$ , a substituted or unsubstituted linear or branched chain alkyl or aryl group; wherein each occurrence of  $R^{iii}$  is hydrogen, CHO,  $COOR^{iv}$ , or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group; and wherein each occurrence of  $R^{ii}$  and  $R^{iv}$  are each independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group.

5. The method of claim 1, wherein each occurrence of  $L^1$  is independently  $-O-(CH_2)_n-$ , wherein n is 0-9, or a glycoside-containing moiety.

6. The method of claim 1, wherein  $L^1$  is  $-O-(CH_2)_n-CH_2-$  and two or more non-adjacent amino acids is/are independently substituted with a moiety having the structure:

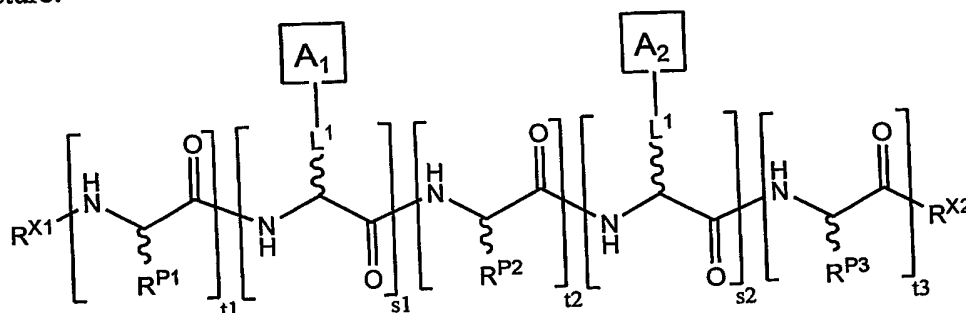


5 wherein each occurrence of  $n$  is independently 0-8.

7. The method of claim 1, wherein each occurrence of A, A1 and A2 is independently selected from the group consisting of Globo-H, fucosyl GM1, KH-1, glycoporphin, STN, (2,3)ST,  $Le^y$ ,  $Le^x$ , N3, Tn, 2,6-STn, Gb3 and TF.

10

8. The method of claim 1, wherein the polyfunctionalized peptide has the structure:



wherein  $s1$  and  $s2$  are independently an integer from 1 to about 20;

15  $t1$ ,  $t2$  and  $t3$  are each independently an integer;

$R^{X1}$  is hydrogen, alkyl, acyl, aromatic, heteroaromatic, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), a nitrogen protecting group, an amino acid or a protected amino acid;

20  $R^{X2}$  is  $-OR^{X2a}$  or  $-NR^{X2b}R^{X2c}$ , wherein  $R^{X2a}$  is hydrogen, alkyl, aromatic, heteroaromatic, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), a carboxylic acid protecting group, an amino acid or a protected amino acid; and  $R^{X2b}$  and  $R^{X2c}$  are independently hydrogen, alkyl, aromatic, heteroaromatic, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), a nitrogen protecting group, an amino acid or a protected amino acid;

$R^{P1}$ ,  $R^{P2}$  and  $R^{P3}$  are independently H, alkyl, heteroalkyl, aromatic, heteroaromatic, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), or a natural or non-natural amino acid side chain;

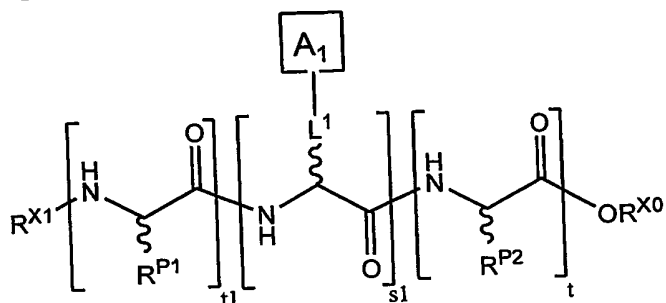
each occurrence of  $L^1$  is independently a substituted or unsubstituted aliphatic or heteroaliphatic moiety;

$A_1$  and  $A_2$  are each independently an aliphatic, heteroaliphatic, aromatic, heteroaromatic, aryl, heteroaryl or a pharmaceutically useful group or entity; and

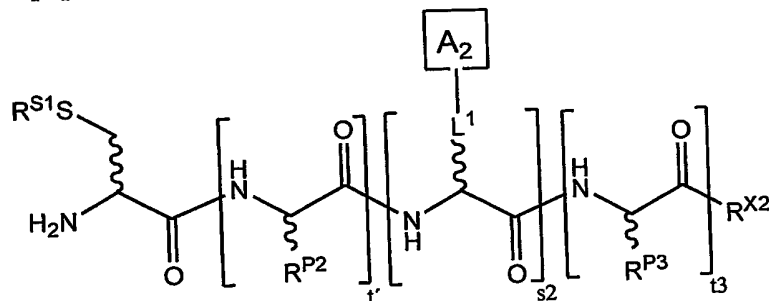
at least one occurrence of the bracketed structure t2 is a cysteine residue or protected cysteine residue;

and the method comprises a step of:

reacting a peptide acyl donor having the structure:



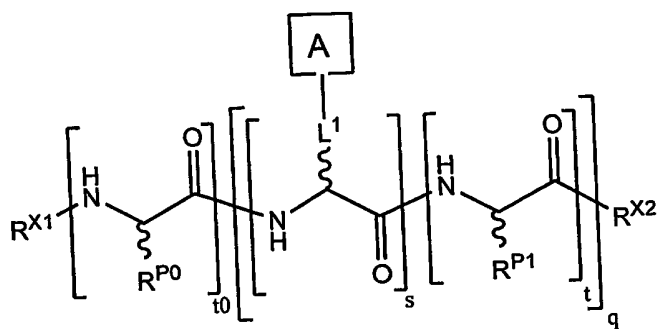
with a peptide amine acceptor having the structure:



under suitable conditions to effect ligation;

wherein the sum  $t+t'$  equals  $(t2)+1$ ;  $R^{S1}$  is a sulfide protecting group; and  $R^{X0}$  is a group such that the moiety  $-C(=O)OR^{X0}$  can be made to undergo ligation with the glycopeptide amine acceptor.

9. The method of claim 8, wherein the step of reacting the peptide acyl donor with the peptide amine acceptor is repeated a desired number of times, to prepare a polyfunctionalized peptide having the structure:



wherein  $R^{X1}$  and  $R^{X2}$  are as defined in claim 8;

each occurrence of A may be the same or different and may be as defined for

$A_1$  and  $A_2$  in claim 8;

5 each occurrence of  $R^{P1}$  may be the same or different and may be as defined for  $R^{P1}$  and  $R^{P2}$  in claim 8;

q is an integer greater than or equal to 2;

each occurrence of s is independently an integer from 1 to about 20;

each occurrence of t is independently an integer;

10  $t_0$  is an integer; and

each occurrence of  $R^{P0}$  is independently H, alkyl, heteroalkyl, aromatic, heteroaromatic, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), or a natural or non-natural amino acid side chain.

15 10. The method of claim 9, wherein q is an integer between 2 and about 5.

11. The method of claim 9, wherein q is 2.

12. The method of claim 9, wherein the sum  $s+t$  is between about 2 and about 6.

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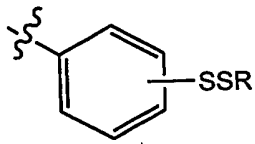
13. The method of claim 9, wherein  $t_0$  is an integer from 0 to about 20.

14. The method of claim 9, wherein  $R^{X1}$  is hydrogen, Fmoc or Ac.

25 15. The method of claim 9, wherein  $R^{X2}$  is  $NH_2$ .

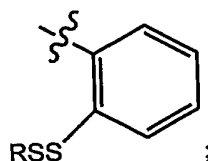
16. The method of claim 9, wherein  $R^{X0}$  is disulfide-substituted aryl moiety.

17. The method of claim 9, wherein  $R^{X0}$  has the structure:



5 wherein R is an aliphatic, heteroaliphatic, aromatic or heteroaromatic moiety.

18. The method of claim 17, wherein  $R^{X0}$  has the structure:

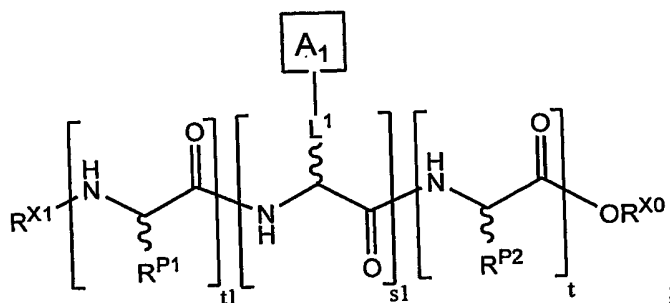


10 wherein R is lower alkyl.

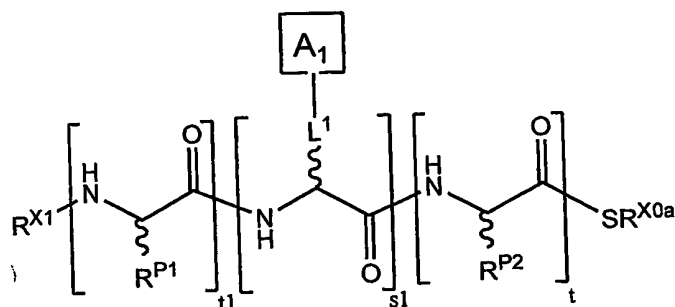
19. The method of claim 18, wherein R is ethyl.

20. The method of claim 9, wherein  $R^{S1}$  is -S/Bu.

15 21. The method of claim 9, wherein in the step of reacting the peptide acyl donor having the structure:



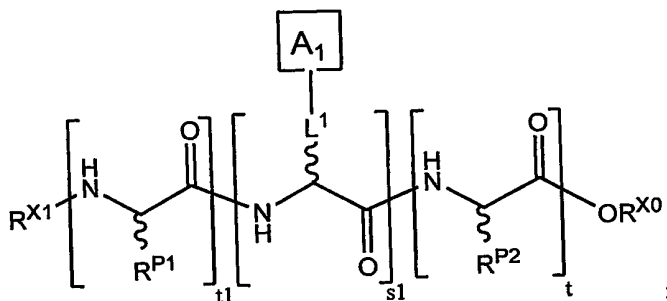
with the peptide amine acceptor under suitable conditions to effect ligation, an intermediate having the following structure is formed in situ:



wherein  $R^{X0a}$  is an oxygen-substituted aryl moiety.

22. The method of claim 21, wherein the suitable conditions to effect ligation  
5 comprise MESNa.

23. The method of claim 9, wherein in the peptide acyl donor having the  
structure:



10 the amino acyl residue directly attached to  $-OR^{X0}$  is phenylalanine.

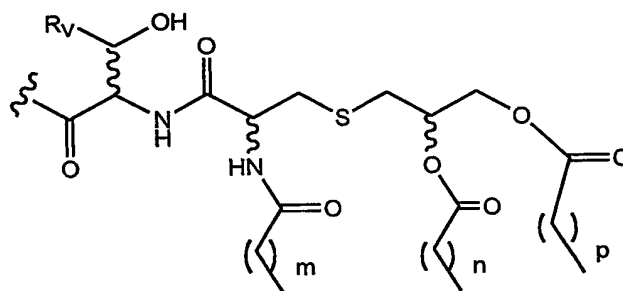
24. The method of claim 1, wherein when at least one occurrence of A (or  $A_1$   
and/or  $A_2$ , as further defined for A) is a carbohydrate domain, some or all of  
carbohydrate domains are O-linked to the peptide backbone.

15 25. The method of claim 1, wherein when at least one occurrence of A (or  $A_1$   
and/or  $A_2$ , as further defined for A) is a carbohydrate domain, some or all of  
carbohydrate domains are N-linked to the peptide backbone.

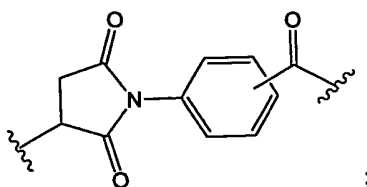
20 26. The method of claim 1, wherein the polyfunctionalized peptide is  
symmetrical.



27. The method of claim 1, wherein the polyfunctionalized peptide is nonsymmetrical.
28. The method of claim 1, further comprising a step of conjugating the polyfunctionalized peptide to an immunogenic carrier.
29. The method of claim 28, wherein the carrier is a protein, a peptide or a lipid.
30. The method of claim 28, wherein the carrier is Bovine Serum Albumin (BSA), Keyhole Limpet Hemocyanin (KLH) or polylysine.
31. The method of claim 28, wherein the carrier is a lipid carrier having the structure:



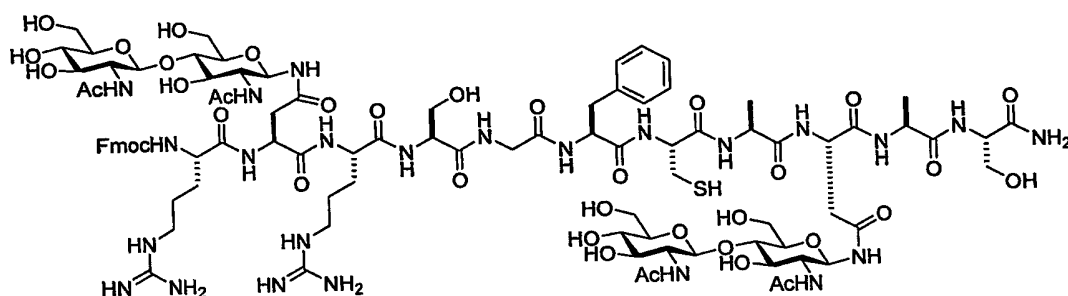
- wherein m, n and p are each independently integers between about 8 and 20; and R<sub>v</sub> is hydrogen, substituted or unsubstituted linear or branched chain lower alkyl or substituted or unsubstituted phenyl.
32. The method of claim 31, wherein m', n' and p' are each 14.
33. The method of claim 28, wherein the carrier is linked to the polyfunctionalized peptide through a crosslinker.
34. The method of claim 33, wherein the crosslinker is a fragment having the structure:



whereby said structure is generated upon conjugation of a maleimidobenzoic acid N-hydroxy succinimide ester with a suitable functionality on the polyfunctionalized peptide.

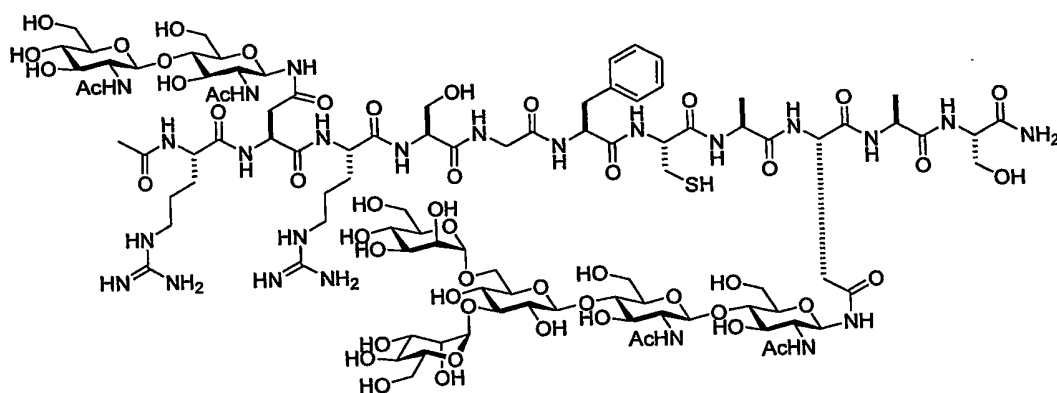
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35. The method of claim 1, wherein the polyfunctionalized peptide has the structure:



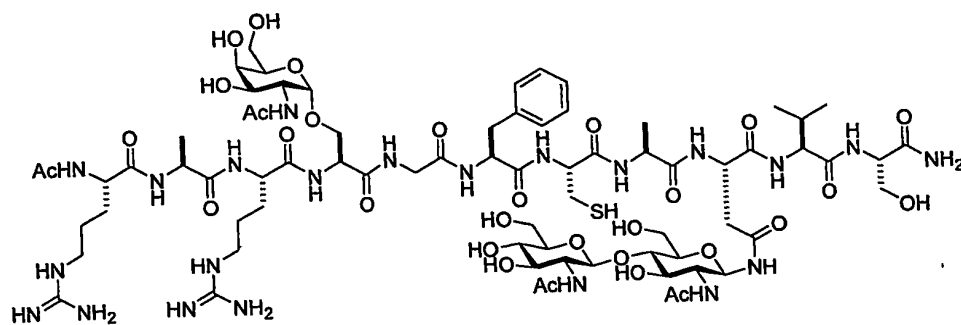
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36. The method of claim 1, wherein the polyfunctionalized peptide has the structure:

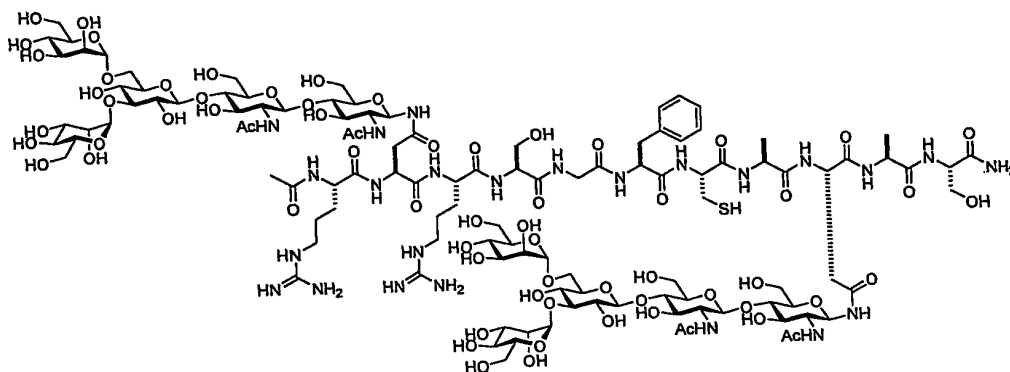


37. The method of claim 1, wherein the polyfunctionalized peptide has the structure:

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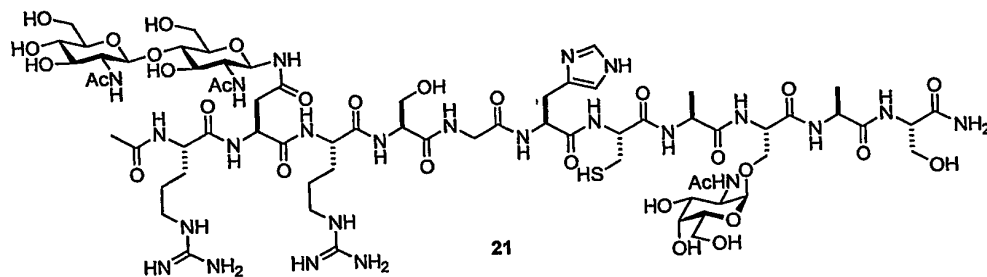


38. The method of claim 1, wherein the polyfunctionalized peptide has the structure:



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39. The method of claim 1, wherein the polyfunctionalized peptide has the structure:



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40. The method of claim 1, wherein the polyfunctionalized peptide has the structure:

